AMENDMENT AND RESPONSE UNDER 37 C.F.R § 1.111

Serial Number:10/664,341

Filing Date: September 16, 2003

Title: RAPIDLY DEGRADED REPORTER FUSION PROTEINS

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IN THE CLAIMS

Please amend the claims as follows:

- 1. (Currently Amended) An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a fusion polypeptide comprising a reporter protein and at least two different heterologous protein destabilization sequences both of which are C-terminal to the reporter protein, wherein one heterologous protein destabilization sequence is SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, or SEQ ID NO:98, wherein another heterologous protein destabilization sequence includes a sequence enriched in proline, glutamic acid, serine, and/or threonine residues (a PEST sequence), wherein the reporter protein is luciferase, fluorescent protein, chloramphenicol acetyltransferase, beta-glucuronidase or beta-galactosidase, and wherein the presence of the at least two different protein destabilization sequences in the fusion polypeptide in a mammalian cell results in enhanced protein degradation relative to a fusion polypeptide with one of the protein destabilization sequences in a corresponding mammalian cell.
- 2. (Canceled).
- 3. (Currently Presented) [[An]] The isolated nucleic acid molecule of claim 1 wherein the eomprising a nucleic acid sequence has eomprising an open reading frame that encodes [[for]] a luciferase and at least one heterologous destabilization sequence, wherein a majority of codons in the open reading frame for the luciferase are codons which are preferentially employed in a mammalian host cell, wherein if one of the heterologous destabilization sequences is 3' to the open reading the nucleic acid molecule further comprises a mRNA destabilization sequence having, the mRNA destabilization sequence is an AU-rich sequence or which forms a stem-loop, and wherein if one of the heterologous destabilization sequences is a protein destabilization sequence, the heterologous protein destabilization sequence is SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ

NO:96, SEQ ID NO:97, SEQ ID NO:98, or a sequence enriched in proline, glutamic acid, serine, and/or threonine residues.

- 4. (Currently Amended) The isolated nucleic acid molecule of claim 1[[, 2]] or 3 further comprising a promoter operably linked to the nucleic acid sequence.
- 5. (Original) The isolated nucleic acid molecule of claim 4 wherein the promoter is a regulatable promoter.
- 6. (Original) The isolated nucleic acid molecule of claim 5 wherein the promoter is an inducible promoter.
- 7. (Original) The isolated nucleic acid molecule of claim 5 wherein the promoter is a repressible promoter.
- 8. (Original) The isolated nucleic acid molecule of claim 1 further comprising a heterologous mRNA destabilization sequence.
- 9. (Currently Amended) The isolated nucleic acid molecule of claim [[2 or]] 8 wherein the mRNA destabilization is 3′ to the nucleic acid sequence.
- 10. (Currently Amended) The isolated nucleic acid molecule of claim 1 [[or 2]] wherein the nucleic acid sequence encoding at least the reporter protein is optimized for expression in a eukaryotic cell.
- 11. (Currently Amended) The isolated nucleic acid molecule of claim 1 [[or 2]] wherein the reporter protein encodes a luciferase.
- 12. (Withdrawn) The isolated nucleic acid molecule of claim 1 wherein the reporter protein encodes a beetle luciferase.

- 13. (Withdrawn) The isolated nucleic acid molecule of claim 12 wherein the reporter protein encodes a click beetle luciferase.
- 14. (Withdrawn) The isolated nucleic acid molecule of claim 1 wherein the reporter protein encodes an anthozoan luciferase protein.
- 15. (Original) The isolated nucleic acid molecule of claim 3 wherein the heterologous destabilization sequence is a protein destabilization sequence.
- 16. (Original) The isolated nucleic acid molecule of claim 3 wherein the heterologous destabilization sequence is a mRNA destabilization sequence.
- 17. (Currently Amended) The isolated nucleic acid molecule of claim 1[[, 2]] or 3 wherein nucleic acid sequence comprises SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, or a fragment thereof that encodes a fusion polypeptide with substantially the same activity as the corresponding full-length fusion polypeptide encoded by SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80.
- 18. (Original) The isolated nucleic acid molecule of claim 1 further comprising a mRNA destabilization sequence.
- 19-20. (Canceled).
- 21. (Withdrawn-Currently Amended) The isolated nucleic acid molecule of claim 1 [[or 2]] wherein one heterologous protein destabilization sequence is from the C-terminus of a

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mammalian ornithine decarboxylase.

22. (Withdrawn-Currently Amended) The isolated nucleic acid molecule of claim 1 [[or 2]]

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wherein one heterologous protein destabilization sequence is a mutant ornithine decarboxylase

sequence.

23. (Withdrawn) The isolated nucleic acid molecule of claim 21 wherein the mutant

ornithine decarboxylase sequence has an amino acid substitution at a position corresponding to

position 426, 427, 428, 430, 431, 433, 434, 439 or 448 of murine ornithine decarboxylase.

24-26. (Canceled).

27. (Withdrawn-Currently Amended) The isolated nucleic acid molecule of claim 1 [[or 2]]

further comprising an ubiquitin polypeptide at the N-terminus of the fusion polypeptide.

28. (Withdrawn) The isolated nucleic acid molecule of claim 27 wherein one of the

heterologous protein destabilization sequences is at the C-terminus of ubiquitin.

29. (Withdrawn) The isolated nucleic acid molecule of claim 28 wherein one of the

heterologous protein destabilization sequences comprises a glutamic acid or arginine residue.

30. (Original) The isolated nucleic acid molecule of claim 10 which encodes a fusion

polypeptide with a half-life of expression of about 20 minutes.

31. (Original) The isolated nucleic acid molecule of claim 10 which encodes a fusion

polypeptide with a half-life of expression of about 30 minutes.

32. (Original) The isolated nucleic acid molecule of claim 15 wherein the heterologous

protein destabilization sequence is a PEST sequence.

- 33. (Withdrawn) The isolated nucleic acid molecule of claim 15 wherein the heterologous protein destabilization sequence is from the C-terminus of a mammalian ornithine decarboxylase.
- 34. (Previously Presented) The isolated nucleic acid molecule of claim 15 wherein the heterologous protein destabilization sequence is SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97 or SEQ ID NO:98.
- 35. (Currently Amended) A vector comprising the nucleic acid molecule of claim 1[[, 2]] or 3.
- 36. (Original) The vector of claim 35 wherein the nucleic acid molecule is operably linked to a regulatable promoter.
- 37. (Original) The vector of claim 36 wherein the promoter is a repressible promoter.
- 38. (Withdrawn) The vector of claim 34 wherein the nucleic acid molecule comprises SEQ ID NO:49, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80 or a fragment thereof that encodes a fusion polypeptide with substantially the same activity as the corresponding full-length fusion polypeptide encoded by SEQ ID NO:49, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79 or SEQ ID NO:80.
- 39. (Withdrawn-Currently Amended) A fusion polypeptide encoded by the nucleic acid molecule of claim 1[[, 2]] or 3.
- 40. (Withdrawn) The fusion polypeptide of claim 38 wherein the reporter protein is chloramphenical acetyltransferase, luciferase, beta-glucuronidase or beta-galactosidase.
- 41. (Original) A host cell comprising the vector of claim 35.

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42. (Original) The host cell of claim 41 which is stably transfected with the vector that

encodes a fusion polypeptide comprising a luminescent protein.

43. (Original) The host cell of claim 42 wherein the signal emitted by the host cell

comprising the vector is greater than the signal emitted by a corresponding host cell comprising a

vector which lacks one or more of the destabilization sequences.

44. (Original) A stable cell line comprising the vector of claim 35 wherein the signal emitted

by the reporter protein is equal to or greater than a signal emitted by a corresponding stable cell

line comprising a vector which lacks one or more of the heterologous destabilization sequences.

45. (Withdrawn) A method to detect a reporter protein in a cell, comprising:

a) contacting a cell with the vector of claim 35; and

b) detecting or determining the presence or amount of the reporter protein in the cell or a

lysate thereof.

46. (Withdrawn-Currently Amended) The isolated nucleic acid molecule of claim 1 [[or 2]]

wherein the reporter protein is green fluorescent protein, chloramphenicol acetyltransferase,

beta-glucuronidase or beta-galactosidase.

47. (Canceled).

48. (New) The host cell of claim 41 which is a mammalian cell.